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## Research Article

# Spasmolytic and Antimicrobial Activities of Crude Extract of *Bongardia chrysogonum* L. Tubers

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## Abstract

**Background and Objective:** Although *Bongardia chrysogonum* L. (family Berberidaceae) has been used in many countries in traditional medicine to treat gastrointestinal tract disorders, scientific evidence for this treatment is lacking. The present study was carried out to assess the antimicrobial activity and to evaluate the antispasmodic potential of *B. chrysogonum* tuber extract.

**Materials and Methods:** Extracts were also evaluated for antimicrobial potential against *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* using the agar well diffusion method. Minimum inhibitory concentration and minimum bactericidal concentration were determined by using the micro dilution method. To evaluate the antispasmodic activity of *B. chrysogonum* extract on contractions induced by the spasmogens acetylcholine, BaCl<sub>2</sub> and KCl, contractions of rat duodenum were recorded using an isolated tissue bath chamber with an isotonic transducer and oscillographic device. Results were compared by one-way analysis of variance (ANOVA) followed by Dunnett's post test. **Results:** Preliminary phytochemical screening of the tuber extract revealed the presence of alkaloids, saponins, tannins and carbohydrates, including monosaccharides. No antimicrobial activity was observed against gram-positive or gram-negative bacteria; however, ethanolic *B. chrysogonum* tuber extract attenuated the basal contractile activity of rat duodenum smooth muscle and decreased contractions induced by acetylcholine, KCl and BaCl<sub>2</sub>, suggesting an antimuscarinic effect and/or interference of calcium influx. Alkaloids and saponins present in the extract may account for this antispasmodic effect, suggesting multiple mechanisms that can be explored in future studies. **Conclusion:** Taken together, results demonstrate that *B. chrysogonum* tuber extract possesses antispasmodic activity but not antimicrobial activity and supports its traditional use to alleviate gastrointestinal spasms.

**Key words:** *Bongardia chrysogonum*, spasmolytic, antimicrobial, phytochemical screening, rat duodenum

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Diarrhea and irritable bowel syndrome are common functional disorders of the intestine that affect millions of people. Abdominal pain and cramps are typical symptoms<sup>1</sup>. In Jordan and other developing countries, medicinal plants are considered effective treatment for gastrointestinal disorders such as diarrhea and irritable bowel syndrome, particularly in rural areas<sup>2</sup>. Although synthetic drugs are available for these disorders, all have side effects that necessitate the search for new drugs.

Traditional medicinal plants have increasing applications and are used to develop medicine with fewer side effects. The antidiarrheal activities of plant extracts can be mediated through spasmolytic effects (intestinal smooth muscle relaxation), delayed gastrointestinal transit, slowed gut motility, increased water absorption or decreased electrolyte secretion, all of which are related to regulation of enteric nervous system<sup>3</sup>. However, scientific studies evaluating these therapeutic claims and elucidating the mechanisms of action are lacking for many of the medical plants used as antidiarrheal treatment in traditional medicine. For example, the ethnopharmacology, chemical properties and pharmacological activities of the Jordanian plant *Bongardia chrysogonum* L. (family Berberidaceae) have not yet been reported. Tubers of this plant are used in traditional medicine to treat epilepsy and gastrointestinal disorders because of their spasmolytic activity<sup>4</sup>. In addition, both rural communities

and researchers describe the effectiveness of these tubers for the treatment of urinary tract infections, hemorrhoids, prostatic hypertrophy, hypercholesterolemia<sup>5,6</sup> and cancer<sup>7</sup>. The tubers are also reported to have potent hypoglycemic effects, making them useful for glucose homeostasis<sup>8</sup>, in addition to multiple anti-inflammatory effects<sup>6</sup>.

*Bongardia chrysogonum*, commonly known as (-Uruf-el-Deek-), is a small perennial plant that is wide spread in the eastern Mediterranean region<sup>9</sup>, Iran, Turkey, Afghanistan and North Africa<sup>10</sup>. The plants have 20-80 cm erect scapes and tuberous rhizomes that are 2-5 cm in diameter. The 1-3 radical leaves are imparipinnate or deeply pinnatisect and petiolate, 10-25 cm long with a petiole about 1/4 as long. The leaves are horizontally spreading, with lateral pinnae in 3-8 opposite pairs and a slightly larger terminal pinna closely subtended by a pair of lateral pinnae (Fig. 1).

Previous studies have reported the presence and isolation of secondary plant metabolites such as alkaloids and triterpenoids<sup>11-15</sup>. However, despite the wide spread use of these tubers, detailed studies have not been conducted to determine their phytochemical composition and biological properties. Therefore, the aim of this research was to determine the phytochemical constituents, antimicrobial activities and possible antispasmodic activity of *B. chrysogonum* (BC) extract. The results may provide support for the traditional use of this plant to treat intestinal disorders. This is the first study to describe antispasmodic activity of this plant.



Fig. 1: *Bongardia chrysogonum* L. plant

## MATERIALS AND METHODS

**Reagents:** Drugs and analytical grade chemicals were purchased from Sigma (St. Louis, MO, USA). All drugs were dissolved in distilled water and dilutions were prepared fresh on the day of the experiment.

**Animals:** Male Wistar rats ( $250 \pm 8.11$  g) were obtained from the Faculty of Medicine, University of Jordan, subjected to a 24 h fast and given access to water *ad libitum* prior to experimentation. All procedures involving animals were carried out in accordance with Jordanian regulations for animal experimentation and care. The protocol was reviewed and approved by the laboratory animal ethics committee of the Scientific Research Council at the Deanship of Academic Research (protocol and ethics approval number UJ 218/2013-2014). The study commenced on December 1, 2014 and lasted for 24 months. All experimentation was carried out at the pharmacology research lab in the Faculty of Medicine. The University of Jordan, Amman, Jordan. All efforts were made to minimize animal suffering and reduce the numbers of animal used.

**Duodenum preparation:** Duodenum preparation was carried out according to the method described by Shatarat *et al.*<sup>16</sup>. After pretreating the tissue with BC extract or control, the cumulative concentration response curve obtained using acetylcholine (ACh) chloride, potassium chloride (KCl) and barium chloride ( $\text{BaCl}_2$ ) was recorded isotonicity and the effect was allowed to reach a steady state at each concentration.

**Plant materials and extraction:** *Bongardia chrysogonum* tubers were collected inspiring in Jordan (15 km of Northwest Amman). The collected material was identified by Professor Suleiman Al-Olimat, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan. A voucher for each plant material has been deposited in the herbarium of the University for Future Reference (BC No. 5450).

Tubers were selected, dried completely under shade and then ground to a fine powder. Crude extracts were prepared by refluxing 500 g of the powdered plant material with 80% ethanol or chloroform for 3 h and maintaining the extract for 5 days at room temperature with continuous shaking (Labtech, Korea). The crude extracts were then passed through 125 mm filter paper (Albet, EEC). The volume of each filtered

solution was increased to 100 mL with solvent and then the solvent was evaporated under reduced pressure using a rotary evaporator (Heidolph Laborota, Germany) to obtain 10% crude extracts ( $100 \text{ mg mL}^{-1}$ ). The extracts were further dried by incubating at room temperature for 8 days. The crude extracts were either used directly or stored in an air-tight container for further use.

**Assessment of antispasmodic activity:** After stabilization for 30 min, the antispasmodic effect of the BC tuber ethanol extract was evaluated by using the following procedures.

**Relaxant effect of BC tuber extract on ACh-induced contractions:** After immersion and equilibration of the duodenum in the organ bath, the dose-response curve was obtained for ACh. An increase in ACh concentration resulted in increased smooth muscle contraction and the submaximal contractile response was obtained at  $3 \times 10^{-5}$  M. This concentration was selected for the cumulative concentration response curves. Increasing concentrations of BC ethanol extract were added to the organ bath 2 min before ACh administration and the effect of the BC extract was expressed as a percentage of the ACh-induced contraction.

**Relaxant effect of BC tuber extract on  $\text{BaCl}_2$ -induced contractions:** Freshly isolated duodenum was also exposed to 5 mM  $\text{BaCl}_2$  to induce contractions. Increasing concentrations of BC ethanol extract were added to the organ bath 2 min before the addition of  $\text{BaCl}_2$  and the effect of the BC extract was expressed as a percentage of the  $\text{BaCl}_2$ -induced contraction.

**Relaxant effect of BC tuber extract on KCl-induced contractions:** To determine  $\text{Ca}^{2+}$  antagonist activity, 60 mM KCl was added to the organ bath to produce sustained contractions. Increasing concentrations of BC ethanol extract were then added to evaluate its inhibitory effects, which were expressed as a percentage of the KCl-induced contractions.

**Solvent effect on rat duodenum contractions:** The effect of the solvent (80% ethanol) on smooth muscle contractions in the isolated rat duodenum was evaluated by adding up to 100  $\mu\text{L}$  ethanol to the organ bath.

**Antimicrobial activity:** The agar well diffusion method was used to evaluate antimicrobial activity of the BC extracts<sup>17</sup> against *Bacillus subtilis* (ATCC 11562), *Staphylococcus aureus*

(ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 29425) and *Pseudomonas aeruginosa* (ATCC 11921). Petri plates were filled with 75 mL seeded agar, which was allowed to solidify. Freshly prepared bacterial inoculum was evenly spread using a sterile cotton swab over the entire agar surface. A 6 mm hole was then punched with a sterile cork borer and 100  $\mu$ L each of ethanolic and aqueous crude BC tuber extract was poured into the well. The Petri plates were allowed to stand at room temperature for 1 h and then incubated at 37°C overnight. The solvent dimethyl sulfoxide (DMSO) was used to fill the well as a negative control and 1 mg mL<sup>-1</sup> norfloxacin was used as a positive control. The zone of inhibition (average diameter) after 24 h was determined for each treatment and compared with the controls.

**Determination of minimum inhibitory concentration and minimum bactericidal concentration:** To confirm the results of the agar diffusion assay, the Minimum Inhibitory Concentration (MIC) of both BC extracts was determined by the microdilution method<sup>18</sup> using nutrient broth. The MIC is defined as the lowest concentration of an antimicrobial that inhibits visible growth of a microorganism after overnight incubation. Plant extracts were dissolved in 10% DMSO and two fold dilutions were prepared with nutrient broth. The nutrient broth containing plant extract was inoculated with 10  $\mu$ L bacterial suspension ( $5 \times 10^6$  CFU mL<sup>-1</sup>), with nutrient broth containing DMSO serving as a negative control. To evaluate bacterial growth, 270 mg resazurin was dissolved in 40 mL sterile water and 10  $\mu$ L of the resazurin solution was added to each sample and incubated for 24 h at 37°C. Bacterial growth was detected by reading absorbance at 500 nm. Bacterial growth was indicated by a color change from purple to pink or colorless (assessed visually). The MIC was defined as the lowest plant extract concentration at which the color changed or the highest dilution that completely inhibited bacterial growth.

The samples were then spread on fresh solid media and incubated for 18 h to determine the Minimum Bactericidal Concentration (MBC) values (i.e., lowest concentration that prevents the growth of an organism). The lowest plant extract concentration that prevented all bacterial growth was reported as the MBC. The MIC and MBC were determined in triplicate experiments.

**Preliminary phytochemical analysis:** Qualitative phytochemical analysis of the ethanolic and chloroform BC tuber extracts was performed according to the standard

procedures described by Harborne<sup>12</sup>, Trease and Evans<sup>18</sup> and Wagner *et al.*<sup>19</sup>. The analysis consisted of the foam test for saponins<sup>12,19</sup>, Liebermann-Burchard test for terpenoids and triterpenoids<sup>18</sup>, FeCl<sub>3</sub> and lead subacetate tests for tannins<sup>12,19</sup>, Shinoda's test for flavonoids<sup>19</sup>, Borntrager's test for anthraquinones<sup>12,19</sup>, Keller-Killiani test for cardiac glycosides<sup>18</sup>, Molisch's test for carbohydrates<sup>12</sup>, Fehling's test for reducing sugars<sup>12</sup>, Barfoed's test for monosaccharides<sup>12</sup>, Millon's test for proteins<sup>12</sup>, ninhydrin test for amino acids<sup>12</sup> and Mayer's test, Dragendorff's test, Wagner's test and 1% picric acid for alkaloids<sup>12,19</sup>. The results are expressed as (+) for the presence and (-) for the absence of the phytochemical.

**Statistical analysis:** Results are expressed as Mean  $\pm$  Standard Error of the Mean (SEM) of 4-6 replicates, as indicated in the figure legends. Results were compared by one-way analysis of variance (ANOVA), followed by the post hoc Dunnett's multiple comparison test<sup>19</sup>,  $p < 0.05$  was considered significant. Statistical analysis was performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA, USA).

## RESULTS

**Effect of BC ethanolic extract on spontaneous contraction in isolated rat duodenum:** In all experiments, the addition of ACh, BaCl<sub>2</sub> (5 mM) or KCl (60 mM) caused a rapid contraction in the duodenum, which returned to the plateau state immediately. This contraction of duodenum (without BC tuber extract) was considered 100%.

**Effect of BC tuber extract on ACh-induced contractions in isolated rat duodenum:** A single application of  $3 \times 10^{-5}$  M ACh evoked a contraction of 100% (Fig. 2), which was inhibited in a concentration-dependent manner by 0.15- 2.5 mg mL<sup>-1</sup> BC tuber extract. This relaxant effect was reversible and the spontaneous activity returned to normal after washing the preparation.

**Effect of BC tuber extract on BaCl<sub>2</sub>-induced contractions of isolated rat duodenum:** The contractile response produced by a single application of BaCl<sub>2</sub> (5 mM) was considered 100% ( $n = 6$ ). The results showed that BC tuber extracts (0.15-2.5 mg mL<sup>-1</sup>) decreased the contractile response evoked by BaCl<sub>2</sub>. No significant decrease was observed with 0.15 mg mL<sup>-1</sup> BC extract; however, contractions were 84.4% of the control ( $p < 0.01$ ) with 0.5 mg mL<sup>-1</sup> extract (Fig. 3).

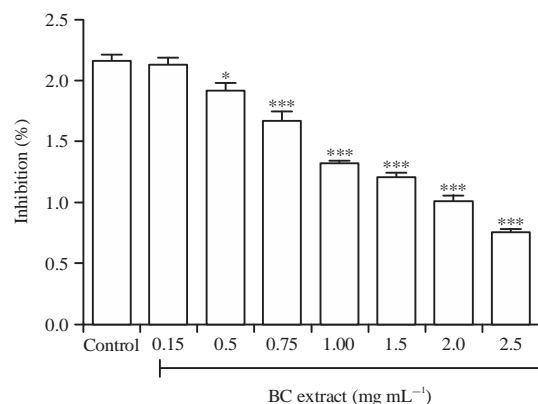


Fig. 2: Concentration-dependent inhibitory effects of crude BC tuber extract on spontaneous contractions and contractions induced by  $3 \times 10^{-5}$  M ACh in isolated rat duodenal smooth muscle

Results are expressed as Mean  $\pm$  SEM (n = 4), \*p<0.01, \*\*\*p<0.001 (ANOVA followed by Dunnett's test, IC<sub>50</sub> = 1.25 mg mL<sup>-1</sup>)

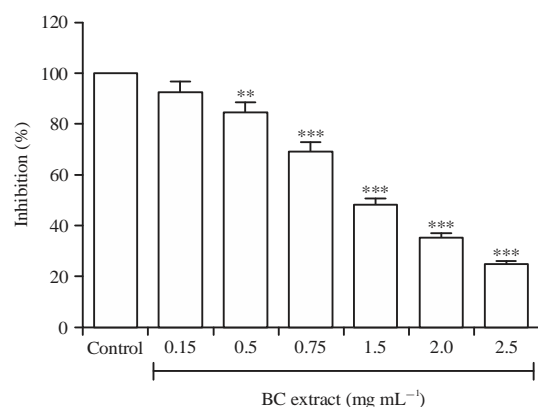


Fig. 3: Concentration-dependent inhibitory effects of crude BC tuber extract on spontaneous contractions and contractions induced by 5 mM BaCl<sub>2</sub> in isolated rat duodenal smooth muscle

Results are expressed as Mean  $\pm$  SEM (n = 6), \*\*p<0.01, \*\*\*p<0.001 (ANOVA followed by Dunnett's test, IC<sub>50</sub> = 1.5 mg mL<sup>-1</sup>)

**Effect of BC tuber extract on KCl-induced contractions in isolated rat duodenum:** The addition of KCl (60 mM) caused sustained contractions (n = 4) in isolated rat duodenum (Fig. 4). The KCl-induced contractions were not significantly affected by 0.15-2 mg mL<sup>-1</sup> BC extract; however, 250 mg mL<sup>-1</sup> BC tuber extract decreased KCl-induced contractile responses to  $53 \pm 6\%$  that of the control.

**Solvent effect on rat duodenum contraction:** To determine whether the solvent used to prepare the BC tuber extract (70% v/v ethanol) could induce smooth muscle contraction,

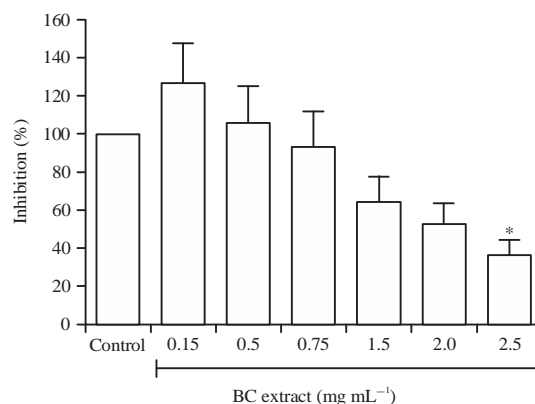


Fig. 4: Concentration-dependent inhibitory effects of crude BC tuber extract on spontaneous contractions and contractions induced by 60 mM KCl in isolated rat duodenal smooth muscle

Results are expressed as Mean  $\pm$  SEM (n = 4), \*p<0.01 (ANOVA followed by Dunnett's test)

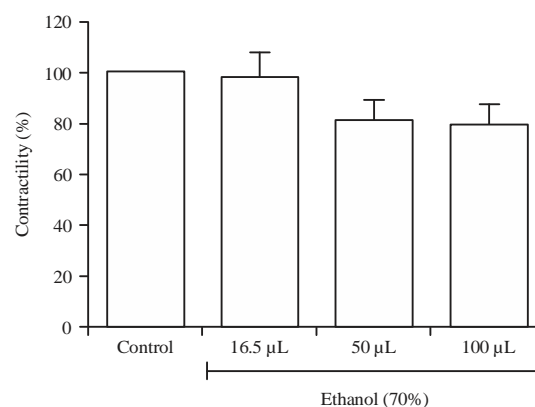


Fig. 5: Effect of 70% ethanol on isolated rat duodenum compared with control (no treatment)

Results are expressed as Mean  $\pm$  SEM (n = 4)

up to 100 µL ethanol was added to the organ bath. Spontaneous rhythmic contractions were not significantly affected by the addition of ethanol (Fig. 5).

**Antimicrobial activity:** The antimicrobial potential of BC was qualitatively evaluated by the presence or absence of inhibition zones and the inhibition zone diameter. The MIC and MBC were determined by using the agar well diffusion method, with norfloxacin serving as a positive control. Results summarized in Table 1 and 2 show that ethanolic BC tuber extract did not inhibit the growth of either Gram-positive or Gram-negative bacteria. Using ethanolic and aqueous tuber extracts, no antimicrobial activity was detected against *B. subtilis*, *S. aureus*, *S. epidermidis*, *E. coli* or *P. aeruginosa*,

Table 1: Antimicrobial activity recorded as zone of inhibition of *Bongardia chrysogonum* extracts against selected bacterial strains, using norfloxacin as a positive control

Microorganisms	Zone of inhibition (mm)		
	Ethanollic extract	Aqueous extract	Norfloxacin
<i>Bacillus subtilis</i>	1.00±0.10	1.0±0.02	25.11±0.40
<i>Staphylococcus aureus</i>	3.00±0.30	2.5±0.03	22.05±0.35
<i>Staphylococcus epidermidis</i>	4.08±0.02	2.0±0.01	26.02±0.20
<i>Escherichia coli</i>	2.04±0.03	0.5±0.01	19.40±0.05
<i>Pseudomonas aeruginosa</i>	1.00±0.00	0.3±0.05	17.11±0.03

Results are expressed as Mean±SEM

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of *Bongardia chrysogonum* extracts against selected bacterial strains, using norfloxacin as a positive control

Microorganisms	Ethanollic extract (µg mL <sup>-1</sup> )		Aqueous extract (µg mL <sup>-1</sup> )		Norfloxacin (µg mL <sup>-1</sup> )	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Bacillus subtilis</i>	620±7.0	800±11	600±7.0	790±10	3±0.4	10±0.05
<i>Staphylococcus aureus</i>	750±2.1	850±19	780±5.2	900±14	1±0.11	3±0.03
<i>Staphylococcus epidermidis</i>	900±7.8	950±4.0	800±7.8	890±4.0	2±0.4	9±0.4
<i>Escherichia coli</i>	820±2.5	850±7.0	855±4.2	820±7.0	5±0.9	15±14
<i>Pseudomonas aeruginosa</i>	950±4.2	980±5.6	900±4.2	890±7.4	20±12	90±18

Results are expressed as Mean±SEM

Table 3: Qualitative phytochemical screening of *Bongardia chrysogonum* tubers

No	Phytoconstituents	Tests/reagents used	Ethanollic extract	Chloroform
1	Carbohydrates	Molisch's test	0	0
2	Reducing sugars	Fehling test	-	-
3	Monosaccharides	Barfoed's test	0	0
4	Proteins	Millon's test	-	-
5	Amino acids	Ninhydrin test	-	-
6	Flavonoids	Shinoda's test	-	-
7	Terpenes/steroids	Liebermann-Burchard's test	-	-
8	Alkaloids	Mayer's test	+++	0
		Dragendorff's test	+++	0
		Wagner's test	+++	0
		Picric acid (1%) test	0	0
9	Cardiac glucoside	Keller-Killani test	-	-
10	Anthraquinones	Borntrager's test	-	-
11	Tannins	Ferric chloride test	0	0
		Lead subacetate test	0	0
12	Saponins	Foam test	+++	-

+++ : Highly present, ++ : Moderately present, + : Slightly present, - : Absence of bioactive compound

suggesting that the plant tuber lacks antimicrobial activity. This is the first study reporting antimicrobial activity of this plant species against selected bacteria strains under these assay conditions.

**Preliminary phytochemical analysis:** Preliminary phytochemical screening of ethanollic and chloroform BC tuber extracts revealed the presence of the following secondary metabolites: Alkaloids, tannins, monosaccharides and other carbohydrates (Table 3). Saponins were detected only in the ethanollic extract. Analysis of the chloroform BC tuber extract showed relatively small levels of alkaloids and

tannins, whereas the ethanollic extract showed relatively high levels of alkaloids, saponins and tannins.

## DISCUSSION

Our results (Fig. 2-5) showed that BC tuber extract was able to not only relax the tone of spontaneous contractions in the isolated rat duodenum but also attenuated the spasmogenic effects of ACh, BaCl<sub>2</sub> and KCL at high concentrations. These spasmogens cause contractions by different mechanisms. The spasmolytic effect of BC tuber extract was reversible and the spontaneous activity returned



to normal after washing the tissue three times. Furthermore, the effect of the ethanolic extract was concentration-dependent.

ACh, KCl and BaCl<sub>2</sub>-induced contractions are commonly used to analyze the spasmolytic activity and/or mechanism of action of drugs and plant extracts *in vitro*. The effects of ACh are receptor-mediated, whereas the effects of KCl are Ca<sup>2+</sup> channel-mediated and BaCl<sub>2</sub> is a nonspecific smooth muscle spasmogen<sup>20-23</sup>. The rhythmic contractions of smooth muscle cells depend on an endogenous pacemaker driven by the cytosolic Ca<sup>2+</sup> oscillator, which is responsible for the periodic release of Ca<sup>2+</sup> from the endoplasmic reticulum. These pulses of Ca<sup>2+</sup> often cause membrane depolarization<sup>24</sup>. The study showed that BC extract inhibits ACh-induced contractions in a dose-dependent manner, suggesting an anticholinergic action. ACh-induced contractions in the rat ileum involve two different mechanisms involving muscarinic receptors<sup>25</sup>. The ACh binds to M<sub>2</sub> and M<sub>3</sub> receptors in the gut tissue, causing smooth muscle contraction by promoting calcium influx via receptor-operated calcium channels<sup>26</sup>. In addition, ACh promotes inositol triphosphate synthesis via phospholipase c activation, which in turn increases calcium release from the sarcoplasmic reticulum<sup>24</sup>. The results showed that ACh alone causes contractions in isolated rat duodenum, and ethanolic BC tuber extract markedly decreased ACh-induced contractions. This result suggests that ethanolic BC tuber extract exerts strong spasmolytic activity by blocking cholinergic receptors, which may therefore be a potential target for BC.

High barium ion concentrations in the extracellular fluid depolarize the smooth muscle membrane and open voltage-dependent calcium channels, resulting in calcium influx<sup>23</sup>. The results of this study showed that BC tuber extract significantly  $p < 0.05$  inhibited BaCl<sub>2</sub>-induced contractions. This inhibitory effect did not involve a decrease in smooth muscle myofilament sensitivity to Ca<sup>2+</sup>, because the effect was not reversed by washing. It is possible that BC tuber extract contains compounds that interfere with Ca<sup>2+</sup> channel activity or with Ca<sup>2+</sup> ion release from intracellular stores.

L-type Ca<sup>2+</sup> channels have been identified in the rat intestine. High K<sup>+</sup> stimulation provokes membrane depolarization and is the most common method used to introduce Ca<sup>2+</sup> into the cell without receptor stimulation<sup>22,23</sup>. It has been reported that high K<sup>+</sup> concentrations (>30 mM) open voltage-dependent calcium channels, causing Ca<sup>2+</sup> influx and smooth muscle contraction<sup>20</sup>. In this study, 60 mM K<sup>+</sup> was used to cause depolarization of the tissue. The addition of BC tuber extract inhibited the K<sup>+</sup>-induced contractions only at the highest concentration tested (2.5 mg mL<sup>-1</sup>).

BaCl<sub>2</sub> depolarizes the smooth muscle membrane and opens voltage-dependent Ca<sup>+</sup> channels, resulting in Ca<sup>2+</sup> influx. The BC tuber extract significantly  $p < 0.05$  inhibited BaCl<sub>2</sub>-induced contractions. This relaxant effect of BC tuber extract on BaCl<sub>2</sub>-induced contractions was concentration-dependent and greater than its effect on KCl-induced contractions. These results suggest that the antispasmodic activity of BC tuber extract may be at least partially mediated by targeting voltage-dependent Ca<sup>+</sup> channels.

Phytochemical studies of the BC tuber demonstrated the presence of alkaloids<sup>11-13</sup> and saponins<sup>14</sup> as the main secondary metabolites, with potential biological activities. Therefore, the spasmolytic activity of BC tuber extract observed in this study may be attributed in part to these metabolites. However, additional studies are needed to test this hypothesis. Antispasmodic activity could be attributed individual bioactive constituents or may be the result of a general inhibitory effect of multiple constituents that inhibit Ca<sup>2+</sup> influx.

Preliminary phytochemical screening is useful to detect bioactive compounds and may lead to drug discovery and development. Results of these tests may also provide evidence for the traditional medical uses of *B. Chrysogonum* and claims about its antispasmodic effects. Future studies are needed to isolate, identify, characterize and elucidate the structure of bioactive compounds of BC.

No detectable antimicrobial activity against gram-positive and gram-negative bacteria was observed for crude BC tuber extract using the agar disk diffusion and microdilution assays. These negative results may be due to the nature of the phytochemical components of the plant and the bacteria used in the antimicrobial tests. The three major chemical constituents of pharmacological interest in the BC tuber extract were saponins, alkaloids and tannins, all of which contribute considerably to its biological activity. Previous studies report that saponins show only weak or no growth inhibition against both gram-positive and gram-negative bacteria (MIC > 000 g mL<sup>-1</sup>)<sup>27,28</sup>. Alkaloids can have potent antimicrobial activities but this effect depends on their chemical nature, their structural class and the type of bacteria tested<sup>29</sup>, which could explain the negative antimicrobial assay results. Tannins have potential as antibacterial compounds when used alone<sup>30</sup> but may be present in insufficient concentrations in the BC tuber extracts to exert antimicrobial effects. In addition, other phytochemicals present in this plant may alter this activity.

No previous studies have published results of phytochemical screening, anti microbial assays or antispasmodic tests of *B. chrysogonum*. Results of this preliminary study indicate the need for future research to

elucidate the bioactive constituents of this plant with respect to the pharmacological activities studied here.

## CONCLUSION AND FUTURE RECOMMENDATION

This study showed that BC tuber extract exhibits significant antispasmodic activity on isolated rat duodenum, providing a scientific basis for the folk medicinal use of *B. chrysogonum* in the treatment of gastrointestinal disorders. Although no antimicrobial activity of the plant extract was detected, phytochemical analysis and published data demonstrate the presence of alkaloids and saponins, which may account for the antispasmodic activity. The phytochemical compounds identified in this study may be responsible for the beneficial effects of *B. chrysogonum*. Extracts from this plant may therefore serve as a good source for useful drugs; however, future research is needed to isolate, purify and characterize the bioactive constituents responsible for the antispasmodic activity of *B. chrysogonum*.

## SIGNIFICANCE STATEMENTS

This study investigated the antimicrobial properties and antispasmodic potential of *Bongardia chrysogonum* L. tuber extract in an isolated rat duodenum model, demonstrating that this extract may have beneficial effects as a novel spasmolytic. This study lends pharmacological credence to the folkloric, ethnomedical use of these plant tubers as a natural remedy for the treatment of abdominal cramps and various gastrointestinal tract disorders.

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